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## Selectins promote tumor metastasis

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**Abstract:** Cancer metastasis is facilitated by cell-cell interactions between cancer cells and endothelial cells in distant tissues. In addition, cancer cell interactions with platelets and leukocytes contribute to cancer cell adhesion, extravasation, and the establishment of metastatic lesions. Selectins are carbohydrate-binding molecules that bind to sialylated, fucosylated glycan structures, and are found on endothelial cells, platelets and leukocytes. There are three members of the selectin family: P-selectin expressed on activated platelets and endothelial cells, L-selectin present on leukocytes and E-selectin expressed on activated endothelial cells. Besides the accepted roles of selectins in physiological processes, such as inflammation, immune response and hemostasis, there is accumulating evidence for the potential of selectins to contribute to a number of pathophysiological processes, including cancer metastasis. Cancer cell interactions with selectins are possible due to a frequent presence of carbohydrate determinants–selectin ligands on the cell surface of tumor cells from various type of cancer. The degree of selectin ligand expression by cancer cells is well correlated with metastasis and poor prognosis for cancer patients. Initial adhesion events of cancer cells facilitated by selectins result in activation of integrins, release of chemokines and are possibly associated with the formation of permissive metastatic microenvironment. While E-selectin has been evaluated as one of the initiating adhesion events during metastasis, it is becoming apparent that P-selectin and L-selectin-mediated interactions significantly contribute to this process as well. In this review we discuss the current evidence for selectins as potential facilitators of metastasis.

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**Cancer cell adhesive interactions – more than just physical attraction**

**Selectins promote tumor metastasis**

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## **Abstract**

Cancer metastasis is facilitated by cell-cell interactions between cancer cells and endothelial cells in distant tissues. In addition, cancer cell interactions with platelets and leukocytes contribute to cancer cell adhesion, extravasation, and the establishment of metastatic lesions. Selectins are carbohydrate-binding molecules that bind to sialylated, fucosylated glycan structures, and are found on endothelial cells, platelets and leukocytes. There are three members of the selectin family: P-selectin expressed on activated platelets and endothelial cells, L-selectin present on leukocytes and E-selectin expressed on activated endothelial cells. Besides the accepted roles of selectins in physiological processes, such as inflammation, immune response and hemostasis, there is accumulating evidence for the potential of selectins to contribute to a number of pathophysiological processes, including cancer metastasis. Cancer cell interactions with selectins are possible due to a frequent presence of carbohydrate determinants – selectin ligands on the cell surface of tumor cells from various type of cancer. The degree of selectin ligand expression by cancer cells is well correlated with metastasis and poor prognosis for cancer patients. Initial adhesion events of cancer cells facilitated by selectins result in activation of integrins, release of chemokines and are possibly associated with the formation of permissive metastatic microenvironment. While E-selectin has been evaluated as one of the initiating adhesion events during metastasis, it is becoming apparent that P-selectin and L-selectin mediated interactions significantly contribute to this process as well. In this review we discuss the current evidence for selectins as potential facilitators of metastasis.

## **Introduction**

Hematogenous metastasis of cancer consists of several steps enabling cancer cells to intravasate, to survive in the blood circulation and to adhere to the vessels, eventually extravasating and establishing new metastatic lesions. Interactions of intravascular cancer cells with blood constituents (platelets, leukocytes) can modulate their capacity to adhere to endothelium in distant organs thereby facilitating metastasis. This process of cancer cell adhesion and seeding to specific vascular beds appears to be a rate limiting step in cancer metastasis. It is well accepted that malignant transformation is associated with abnormal glycosylation resulting in presentation of altered carbohydrate structures. Enhanced expression of sialyl Lewis<sup>x</sup> and sialyl Lewis<sup>a</sup> structures is frequently associated with cancer progression and poor prognosis [1]. At the same time, these carbohydrates are recognized selectin ligands. Selectins are vascular cell adhesion molecules involved in interactions of leukocytes, platelets and endothelium. Due to the temporal and spatial presence of selectins during hematogeneous dissemination and early organ colonization, interactions with carbohydrate ligands on cancer cells can be expected. Studies from several laboratories suggest that metastatic cancer cells exploit similar mechanisms as used by leukocytes for adhesion and extravasation through endothelium that may contribute to metastasis [2, 3]. Recent evidence also indicates that selectin-mediated interactions may contribute to formation of a permissive microenvironment for metastasis [4].

## **Selectins – vascular cell adhesion molecules**

Selectins were identified to be the key adhesion molecules that mediate leukocyte trafficking and hemostasis, soon after their cloning and characterization [5-7].

Subsequently, selectins were implicated in many human diseases including cancer [3, 7-10]. Selectins are type-I transmembrane glycoproteins consisting of an extracellular C-type lectin domain, an EGF domain, two to nine consensus repeats, a transmembrane domain and a cytoplasmic tail [5]. L-selectin is constitutively expressed on all myeloid cells, on naïve T-cells and some activated memory T-cells [5, 11]. Activation of leukocytes leads to a rapid loss of L-selectin from their cell surfaces due to a proteolytic cleavage [7]. P-selectin is constitutively expressed in endothelial cells of the lung and the choroid plexus, megakaryocytes, thrombocytes and is stored within Weibel-Pallade bodies of endothelial cells or alpha-granules of platelets, respectively [5, 6]. P-selectin is rapidly presented on the surface of activated endothelial cells or platelets by means of exocytosis of storage granules and their fusion with the cell membrane [12-14]. E-selectin is expressed exclusively by endothelial cells, mainly in response to local activation [5]. Constitutive expression of E-selectin has been detected in the skin and parts of bone marrow microvasculature [15]. In most vessels *de novo* synthesis and expression of E-selectin can be induced within two to six hours in response to inflammatory stimuli (e.g. TNF- $\alpha$  and IL-1 $\beta$ ) or disturbed blood flow [5, 16]. Expression of P- and E-selectin is tightly regulated during homeostasis, thereby ensuring spatial and temporal adhesion/recruitment of leukocytes. However, E-selectin may remain up-regulated in the endothelium in areas of chronic inflammation [17, 18].

### **Selectin ligands**

Selectin-based adhesion is mediated by heterotypic interactions of their C-type lectin domain with glycan-bearing ligands. Functional selectin ligands require post-

translational modification of scaffold proteins by glycosyltransferases and sulfotransferases [5, 19-21]. The minimal recognition motif for all selectins is the sialyl-Lewis<sup>x</sup> sLe<sup>x</sup> and its isomer sialyl-Lewis<sup>a</sup> (sLe<sup>a</sup>) tetrasaccharide that is synthesized by  $\alpha$ 1,3-Fucosyltransferases IV or VII,  $\alpha$ 2,3-Sialyltransferases,  $\beta$ 1,4-Galactosyltransferases and N-Acetyl-Glucosaminyltransferases [5, 21, 22]. Efficient selectin-binding to carbohydrates usually requires a glycoprotein scaffold that facilitates the presentation of several selectin ligands in clusters [5, 20]. P-selectin glycoprotein ligand-1 (PSGL-1) is the best characterized ligand, which is concentrated on the tips of microvilli on leukocyte cell surfaces. All three selectins bind to PSGL-1, while potent binding of P- and L-selectin to PSGL-1 is facilitated by additional sulfation of the amino-terminal part of the protein [5, 6]. Although leukocytes express a number of different selectin ligands, e.g. CD44 [23], or E-selectin ligand-1 (ESL-1) [24], PSGL-1 is the main physiological ligand for P-selectin [5, 6]. L-selectin mediates the trafficking of leukocytes, in particular lymphocytes through binding to peripheral node addressins (PNAds) that are endothelial glycoproteins [21]. E-selectin binds to a number of ligands, including PSGL-1, ESL-1, CD44, CD43,  $\beta$ 2-integrins, and L-selectin [5, 25-29]. Mucins are major carriers of altered glycosylation that occur during progression of epithelial cancers - carcinomas. Mucins are high-molecular-weight molecules containing a protein core substituted with a large number of O-linked glycan structures [9, 30]. Major alterations are an enhanced expression of sLe<sup>x/a</sup>, or Tn and sialyl-Tn antigen structures on mucins [31]. Carcinoma mucins expressing sLe<sup>x/a</sup> structures are major selectin ligands on cancer cells. In addition to mucins, several other selectin ligands on cancer cells have been described including CD24, CD44, death-receptor 3, ESL-1 and PSGL-1 [32-35]. However, the *in vivo* relevance of these ligands remains to be determined [24]. Finally, P- and L-selectin,

but not E-selectin can bind to sulfated glycans including heparin, heparin sulfate, fucoidan and sulfated glycolipids in a calcium-independent manner [20, 36]. The large variety of different carbohydrate structures recognized by selectins indicates that the lectin domain of selectins recognize a certain carbohydrate “patch” that can be generated in different ways [20].

### **Roles of selectins health and disease**

The characterization of selectin and selectin ligand deficient mice has provided information about the physiological functions of selectins in inflammation, immune response, wound repair and hemostasis [37-41]. These findings have been further confirmed in mice deficient in selectin ligands, e.g. FucT-VII, Core 2 glucosaminyltransferase, or PSGL-1 [42-45]. Extravasation of leukocytes comprises sequential steps including tethering of leukocytes on endothelial cells, fast and slow rolling, firm adhesion and trans- or para-endothelial migration [46, 47]. Selectins mediate the initial tethering and are critically involved in the transition from fast to slow rolling before leukocytes firmly adhere to endothelial cells [5, 6, 47]. Transition from fast to slow rolling requires activation of integrins that is in part supported by selectin or PSGL-1 outside-in signaling [48, 49]. Given the central role in leukocyte extravasation and trafficking, selectins are involved in innate and adaptive immune responses and this topic is extensively covered elsewhere [5, 21, 47]. Importantly, cell homing studies in mice showed that adhesion of leukocytes differs between organs and types of inflammation [7, 50].

P-selectin on platelets is involved in thrombus formation during hemostasis [51, 52]. The procoagulant activity of P-selectin is mainly explained by its role in the formation of

leukocyte derived microparticles containing tissue factor, the key inducer of the extrinsic coagulation cascade [52, 53]. Moreover, PSGL-1 on platelets could enhance interactions between them and mediate binding of platelets to P- and E-selectin on activated endothelial cells, thus influencing hemostasis [54].

Acute systemic inflammatory responses to infectious or toxic stimulants and trauma are uncontrolled inflammatory, life threatening events that can lead to SIRS (systemic inflammatory response syndrome), sepsis and eventually to multi-organ failure [55]. Selectins are involved in acute organ damage during sepsis by mediating the recruitment of myeloid cells [56]. In different disease models, P- or E-selectin deficient mice are protected from acute inflammation and tissue damage [57, 58]. L-selectin deficiency also decreases leukocyte recruitment to sites of acute inflammation [59]. Inhibition of E- or P-selectin by antibodies or heparin showed significantly reduced disseminated intravascular coagulation and mortality in an experimental septic shock model [60].

Chronic inflammation plays a key role in atherosclerosis, the main cause of morbidity and mortality in developed nations [61]. Recruitment of inflammatory cells during progression of the atherosclerotic lesion is partially selectin dependent [61]. The absence of P-selectin reduces atherosclerotic lesions due to reduced monocyte recruitment [62]. Monocyte recruitment during atherogenesis is facilitated by P-selectin on activated platelets adherent on the endothelium [63]. P-selectin mediated rolling of platelets on activated endothelial cells further supports progression of atherosclerotic lesions [64]. Atherosclerosis is partly decreased by the absence of E-selectin [65]. Finally, L-selectin was recently implicated in atherosclerosis by enhancing lymphocyte recruitment [66].



### **Selectin interactions beyond cell adhesion**

Selectin-mediated interactions facilitate not only cell adhesion, but may also participate in signal transduction, thereby affecting cell migration and activation of other adhesion molecules including integrins [47-49, 67]. Selectin engagement can trigger signals in both the selectin-expressing and the ligand-expressing cells [68, 69]. While the signaling pathways for selectins are just being revealed, leukocyte extravasation represents the best described selectin-initiated signaling pathway [47]. Binding of L-selectin can activate leukocytes and affect the cytoskeleton through interactions between the cytoplasmic tail of L-selectin with  $\alpha$ -actinin, ezrin/radixin/moesin (ERM) family proteins, calmodulin, increase of intracellular calcium and induction of the p38 mitogen-activated kinase pathway [49, 70-74]. L-selectin mediated activation of neutrophils can lead to the production of reactive oxygen species, TNF- $\alpha$ , interleukin-8 and integrins, including CD11b [49, 75]. Monocytes can be activated by the engagement of PSGL-1 on their surfaces by P-selectin [76]. P-selectin binding to PSGL-1 induces signaling that activates integrins through the Src kinase family, Nef-associated factor 1 and phosphoinositide-3-OH-kinases [48, 77, 78]. The cytoplasmic tail of PSGL-1 also interacts via ERM proteins with the cytoskeleton and the spleen tyrosine kinase (SYK) that may transmit signals [79, 80]. There is also evidence that the engagement of P-selectin transmits signals to endothelial cells and platelets, while the mechanism remains unclear [49]. E-selectin binding to PSGL-1 activates  $\beta$ 2 integrins through the SYK and Src kinase pathway that is required for initiation of neutrophil slow rolling [81]. E-selectin itself can transduce signals into endothelial cells through p38 and p42/p44

MAPK pathway [69]. Finally, selectin binding to cell-surface-bound mucins may also transmit signals into cancer cells during cancer progression, e.g. by enhancing growth factor receptor signaling [30, 82].

### **Selectins and cancer progression**

How cancer cells metastasize is still not completely understood. Experimental evidence and observational clinical studies suggest that cancer cell seeding requires specific interactions with the local microenvironment that lead to tumor cell adhesion or mechanical arrest, survival, extravasation and outgrowth in target organs. As described above, the majority of cancer cells, carcinoma cells in particular, express elevated levels of selectin ligands that are associated with poor prognosis [reviewed in 1, 10, 31]. Selectin-mediated interactions of cancer cell binding to and activation of leukocytes, platelets and endothelial cells provide a mechanistic explanation for this poor prognosis (Figure 1) [83-85]. Cell-cell interactions with leukocytes, platelets and endothelium appear to be critical for metastatic progression. Parallels between selectin-mediated leukocyte recruitment and extravasation during inflammation, and adhesion/arrest and extravasation of cancer cells during metastasis has been observed in different models (Figure 1) [2, 8, 9]. Moreover, recent findings suggest that selectins mainly support tumor progression via the cooption of inflammatory pathways [4]. Finally, selectins are strongly involved in coagulation abnormalities often observed in cancer patients, further affecting the progression of the disease [10, 86, 87].

## **E-selectin during cancer progression**

E-selectin is a major adhesion receptor on endothelial cells for leukocytes and has been repeatedly shown to support metastasis *in vivo* [88, 89]. E-selectin has been investigated as a mediator of the process by which cancer cells adhere/arrest in the microvasculature during metastasis [3, 90, 91]. The presence of E-selectin ligands on cancer cells correlates with enhanced adhesion to activated endothelium [92-95]. E-selectin ligands on cancer cells have been found to be mostly mucins [96], yet several unique E-selectin ligands have been recently identified on cancer cells, including death receptor-3 and a specific CD44 glycoform [33, 34]. E-selectin binding of death receptor 3 or other ligands on cancer cells has been shown to increase survival during metastasis [34, 97]. Recent work provides evidence that E-selectin binding to cancer cells may alter the gene expression profile of cancer cells [97]. Furthermore, E-selectin has been shown to regulate endothelial transmigration through activation of p38 and ERK AP kinases [98, 99]. Since distinct E-selectin ligands on neutrophils have been shown to induce different reactions [24], it can be expected that the nature of E-selectin ligands presented on cancer cells may induce specific processes both in endothelial cells as well as in cancer cells.

E-selectin is expressed on activated endothelial cells and has been shown to be upregulated during metastatic colonization of the liver [88, 89]. Downregulation of E-selectin expression or inhibition of E-selectin function resulted in attenuation of experimental liver metastasis. In a transgenic mouse model over-expressing E-selectin in the liver, metastases were redirected to this organ, thus confirming the role of E-selectin in this process [100]. In an experimental metastasis model where mice were injected with IL-1, co-injection of cancer cells with soluble E-selectin probe led to a

reduction of metastasis in the lungs [93]. However, induced E-selectin expression by a high cytokine dose does not reflect the natural process of metastatic lung colonization. We recently demonstrated that E-selectin is dispensable in a model of experimental lung metastasis [101]. These findings probably reflect organ specific differences in the colonization process and different requirements for selectin-mediated interactions depending on the primary tumor and the metastatic organ. Nevertheless, *in vivo* evidence for cancer cell rolling on activated endothelium mediated through E-selectin that leads to metastatic colonization requires further investigation.

### **P-selectin during cancer progression**

The close “relationship” of circulating cancer cells with platelets is one of the factors contributing to cancer progression [14, 102-104]. Substantial evidence indicates that platelets interact with circulating cancer cells, thereby creating tumor microemboli that may facilitate arrest in distant organs and subsequently promote interactions with endothelium [102, 103]. Evidence that platelets support metastasis initially came from models of experimentally induced thrombocytopenia [105, 106]. Likewise, platelet-deficient mice lacking Nf-E2 showed reduced metastasis [107]. The underlying mechanism for the initiation of platelet-cancer cell thrombi formation remains to be defined. However, experimental evidence strongly indicates that platelet P-selectin contributes to the thrombi formation and aids colonization of cancer cells in the lungs [84, 108]. Platelet aggregates on cancer cells lodged in the vasculature was virtually absent in P-selectin deficient mice and was associated with a limited initial seeding of cancer cells to the lungs [108]. Platelets were shown to bind to cancer cells through carcinoma cell surface mucins in a P-selectin dependent manner. Removal of cell

surface mucins from cancer cells prior to intravenous injection resulted in attenuation of metastasis [108]. Bone marrow transplantation experiments in P-selectin deficient mice have shown that endothelial P-selectin also contributes to metastasis [109]. Furthermore, inhibition of P-selectin-mediated interactions of platelets with cancer cell ligands by heparin treatment attenuates cancer metastasis in mice [108, 109].

Patients suffering from advanced cancer are at high risk for thromboembolic events, a finding initially described by Trousseau [87]. The particular association between thrombotic diathesis and mucinous carcinomas led to hypothesis that mucins, carrying selectin ligands, trigger this syndrome. Intravenous injection of purified carcinoma mucins led to a rapid generation of platelet-rich microthrombi [87]. Interestingly, in P-selectin but also L-selectin deficient mice the microthrombi generation was markedly diminished, indicating the involvement of platelet P-selectin and leukocytes in thrombotic events accompanying cancer progression.

### **L-selectin during cancer progression**

Initial studies focused on the potential role of L-selectin on tumor cells by homing to L-selectin ligand expressing tissues [110, 111]. Transgenic ectopic expression of L-selectin in a spontaneous mouse carcinoma model facilitated lymph node metastasis [110]. However, naturally occurring L-selectin on a B-cell lymphoma cell line was not associated with enhanced lymph node metastasis [111].

Inflammation is a key component of the tumor microenvironment and leukocytes have been shown to promote both primary tumor growth as well as metastatic dissemination [112, 113]. Leukocyte responses to cancer are not unique, and have many parallels with the inflammatory response to infection and during wound healing [112, 114].

Contribution of leukocytes to metastasis largely depends on spatial and temporal stimulation that is defined by the microenvironment and cancer cells [112, 114, 115]. L-selectin-mediated leukocyte recruitment to locally activated endothelial cells is one possible means that may be exploited by cancer cells. The contribution of L-selectin to metastasis was analyzed in L-selectin deficient mice [83, 85]. The absence of L-selectin led to attenuation of metastasis. This finding actively implicates leukocytes to the process of metastasis, since L-selectin expression is restricted to leukocytes [83]. L-selectin mediated recruitment of leukocytes to tumor emboli was found to occur subsequent to P-selectin-mediated platelet-cancer cell complex formation [85, 101]. These observations suggest that L-selectin mediates recruitment and adhesion of leukocytes within the microenvironment of metastasizing cancer cells, either with the tumor thrombus itself or with the adjacent endothelium. Concomitantly, enhanced expression of L-selectin ligands around the cancer cell embolus correlated with the recruitment of leukocytes to the intravascular cancer cells, indicative of locally activated endothelium [85]. Leukocytes associated with cancer cells in the vasculature were identified as CD11b positive cells of myeloid origin (monocytes, neutrophils, or precursors). Further studies have shown that monocytes can directly increase transendothelial migration of cancer cells through L-selectin mediated interactions (L.B., manuscript in preparation). Thus, leukocytes may assist cancer cells in breaching the endothelial barrier at sites of intravascular arrest, thereby facilitating metastatic colonization [116].

## **Selectins promote metastasis by shaping the metastatic niche**

There is sufficient experimental evidence that selectins mainly promote metastasis by facilitating heterotypic interactions between cancer cells and blood components including endothelial cells (Figure 2) [3, 10].

After entering the bloodstream directly or via lymphatics, cancer cells are transported to distant organs, where they are arrested within the microvasculature (Figure 2a) [117, 118]. Platelet-cancer cell association and the formation of a tumor embolus during the intravascular phase is thought to be a key step during metastatic dissemination and colonization (Figure 2b) [14, 102-104, 119]. Cancer patients with advanced, metastatic disease have increased serum levels of markers of platelet activation and a higher percentage of activated platelets, probably indicating platelet activation during metastatic spread [104, 120]. Whether cancer cell-platelet interactions are directly initiated after intravasation or after microvascular arrest of cancer cells remains unclear. In experimental metastasis models, formation of tumor emboli within the pulmonary microvasculature is observed rapidly after the injection of cancer cells (Figure 2b) [105, 108]. Nevertheless, the proof for significant tumor cell emboli formation in cancer patients is hampered by pre-analytical treatment of blood samples with a considerable chance of introducing artifacts. Since the isolation methods for circulating cancer cells are becoming more standardized [121, 122], the isolation of circulating tumor emboli may become feasible. Primary activation of platelets by cancer cells *in vivo* is not yet completely understood, but abundant evidence indicates the involvement of tissue factor induced formation of thrombin and signaling through protease activated receptors (PAR) [123, 124]. In mice with platelets lacking PAR4, activation of platelets was strongly reduced and metastasis diminished [107]. Besides minor roles for GPIIb/IIIa and

GP1b/IX in aggregation of platelets on cancer cells [105, 125-127], tumor emboli formation mainly depends on P-selectin mediated interactions [108]. Platelets and tumor emboli formation may promote metastasis by various means including protection from immune-mediated clearance of cancer cells through NK-cells [128, 129], enhancement of microvascular arrest [127], cancer cell invasion through secretion of proteases [130, 131], as well as enhanced angiogenesis and growth through the release of various cytokines such as PDGF and VEGF and bioactive lipids like lysophosphatidic acid [14, 104, 132]. We recently provided evidence that platelets promote metastatic colonization by supporting the formation of an inflammatory niche within the lung microvasculature [4]. P-selectin mediated interactions between cancer cells, platelets and neutrophils led to a local inflammatory activation of endothelial cells [4]. Platelets can support inflammatory activation by the release of cytokines and chemokines [13]. It is conceivable that the formation of a tumor embolus through P-selectin leads to locally increased levels of platelet cytokines and chemokines. Platelet aggregation and tumor emboli formation may additionally occlude microvessels and thus lead to hypoxic areas, promoting a local inflammatory reaction. Moreover, platelet aggregation on cancer cells induces and is perpetuated by the coagulation cascade, finally resulting in increased levels of thrombin and fibrin deposition [119]. Thrombin itself can activate cancer cells and the local endothelium through PAR signaling [119, 124], thereby potentially enhancing local inflammation.

The inflammatory microenvironment that is generated by tumor-embolus formation activates the adjacent endothelium and recruitment of leukocytes (Figure 2c). Markers of endothelial cell activation and inflammation are up-regulated during the first hours after microvascular arrest of cancer cells in models of experimental liver and lung metastasis



[88, 101, 133]. Endothelial cell activation markers such as soluble E-selectin and ICAM-1 detected in plasma are elevated in cancer patients with advanced disease [134]. Reduction of endothelial cell activation in experimental models was associated with decreased metastasis [135, 136]. Activated endothelial cells further support local inflammatory activation and formation of a metastatic niche, mainly by mediating the recruitment of leukocytes. Recruitment and activation of inflammatory cells, in particular myeloid cells are associated with enhanced metastatic colonization [4, 85, 116, 137, 138]. Selectin mediated interactions facilitate the recruitment of leukocytes to the metastatic microenvironment [4, 83, 85]. L-selectin mediated interactions were demonstrated to be essential for the recruitment of neutrophils and monocytes in an experimental model of metastasis [83, 85]. Interactions of L-selectin with a yet unidentified FucT VII dependent ligand on activated endothelial cells contribute to recruitment of leukocytes [85]. Furthermore, selectin dependent interactions between cancer cells, platelets and neutrophils led to the up-regulation of endothelial CCL5 and subsequent recruitment of monocytes to the metastatic microenvironment [4]. Inhibition of CCL5 and subsequently reduced monocyte recruitment attenuated metastasis [4]. Myeloid cells may support metastatic colonization by local immune-suppression, cancer cell survival, invasion and extravasation (Figure 2d) [114, 139, 140]. Monocytes and descendant macrophages were recently shown to facilitate lung colonization by enhancing extravasation [116]. L-selectin dependent recruitment of monocytes can directly increase vascular permeability and transendothelial migration of cancer cells (L.B., manuscript in preparation). E-selectin has also been shown to contribute to the extravasation process [91].

Many studies linking selectins to the metastatic process have utilized experimental metastasis models. A limitation of direct intravascular injection of cancer cells is the absence of a pre-metastatic niche. The pre-metastatic niche was first identified in mouse models of spontaneous metastasis and has subsequently been shown to be present in cancer patients [141-143]. Myeloid precursor cells are mobilized and recruited to specific locations by the primary tumor in an endocrine dependent manner [141-143]. Thereby myeloid cells can shape the local microenvironment in order to make it permissive for arriving cancer cells [141-143]. The role of selectins in the formation of the pre-metastatic niche, however, remains largely elusive and necessitates further studies and validation in human cancer patients.

### **Selectin-inhibitors as anti-metastatic therapeutics**

The expression and involvement of selectins during inflammation, immune response and cancer led to the rationale that inhibitors of selectins could be used as anti-inflammatory, immune-modulating, and anti-cancer agents and several types of inhibitors were developed over the past years [7, 144]. Selectin inhibitors can be divided into glycan-based molecules, small molecules including glycomimetics, soluble forms of ligands and antibodies either targeting selectins or their ligands. The first tested inhibitor was a sialyl-Lewis<sup>x</sup> tetrasaccharide that could target all selectins. However the low affinity, high production costs and unfavorable pharmacokinetics made it not suitable for further development [7]. Glycometabolic inhibitors have been developed that indirectly reduce the production of selectin ligands by inhibition of relevant glycosyltransferases, in particular O-glycosylation of mucins and fucosyltransferases [144, 145]. Treatment of cancer cells with a disaccharide-based inhibitor that serves as a decoy for the glycan

synthesis led to reduction of cancer cell-associated sLe<sup>x</sup> structures and subsequently to attenuation of metastasis [146, 147]. A recombinant soluble form of PSGL-1 was shown to inhibit the rolling of leukocytes *in vivo*, and prevents inflammation in different modes of inflammation [144, 148, 149]. However, clinical trials were discontinued partly due to high production costs [7]. Small molecules or glycomimetics including bimosiamose (TBC-1269), cylexin (CY1503) and efomycine M that inhibit selectins were tested in models of ischemia-reperfusion injury, psoriasis and asthma and to some extent evaluated in clinical trials [144, 150-152]. Selectin blocking antibodies that successfully passed preclinical evaluation were examined in patients with psoriasis, reperfusion injury or cerebral ischemia with no or only minimal clinical effect [151].

Heparin is a glycosaminoglycan exclusively expressed and stored in mast cells and consists of N- and O-sulfated alternating galactosamine/glucosamine and glucuronic acid/iduronic acid moieties [153]. Heparin and fractionated low molecular weight heparins are clinically used for their anti-coagulant activity via antithrombin III and the pentasaccharide sequence for this effect is well characterized [153]. Moreover, unfractionated heparin and some clinically used low molecular heparins bind to P- and L-selectins and inhibit their function [108, 154, 155]. However, not all low molecular weight heparins show inhibitory activity on selectins, indicating that selectin binding to heparin requires a specific properties independent of anticoagulant activity [155, 156]. Indeed, chemically modified heparins with minimal anticoagulant activity but retained its selectin inhibitory activity efficiently attenuated experimental metastasis [10, 108, 157-159]. Besides inhibition of selectins, heparins probably improve survival by influencing heparanase activity, growth factor signaling and inhibition of different cell adhesion

molecules including integrins [36]. In clinical trials, heparins are associated with a better survival of patients [160].

Selectins are mainly implicated in hematogenous metastasis during the intravascular phase of cancer cells. Based on the above described mechanism, heparin inhibition of P- and L-selectin should be applied immediately following initial cancer diagnosis until a period of time (e.g. a few weeks) after surgical removal of the primary tumor [161]. Moreover, the demonstrated implication of selectins in the metastatic process warrants the application of selectin inhibitors to patients with a local tumor stage in order to prevent dissemination. Since selectins are crucial for homeostasis, it is unlikely that prolonged treatment will be feasible. Based on the proposed treatment window encompassing about two months, the specific inhibition of one or two selectins should be well tolerated. Interestingly, there is precedence for the prolonged inhibition of P-selectin. Therapeutic doses of heparin efficiently inhibit P-selectin and also L-selectin, therefore cancer patients treated with heparin as an anticoagulant inadvertently achieved significant P-selectin inhibition [154]. Clinical trials are currently ongoing that evaluate the use of low molecular weight heparins after surgery of localized tumors in addition to adjuvant systemic treatment (e.g. NCT00475098, NCT00967148). Finally, modified heparins with a selective effect on selectin inhibition or other inhibitors of selectins require further examination in preclinical models and testing in early clinical trials.

### **Conflict of interest**

None

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## References

1. Kannagi R, Izawa M, Koike T, Miyazaki K, Kimura N. Carbohydrate-mediated cell adhesion in cancer metastasis and angiogenesis. *Cancer Sci* 2004;95:377-384.
2. Krause T, Turner GA. Are selectins involved in metastasis? *Clin Exp Metastasis* 1999;17:183-192.
3. Witz IP. The selectin-selectin ligand axis in tumor progression. *Cancer Metastasis Rev* 2008;27:19-30.
4. Läubli H, Spanaus KS, Borsig L. Selectin-mediated activation of endothelial cells induces expression of CCL5 and promotes metastasis through recruitment of monocytes. *Blood* 2009;114:4583-4591.
5. Kansas GS. Selectins and their ligands: current concepts and controversies. *Blood* 1996;88:3259-3287.
6. McEver RP. Selectins: lectins that initiate cell adhesion under flow. *Curr Opin Cell Biol* 2002;14:581-586.
7. Ley K. The role of selectins in inflammation and disease. *Trends Mol Med* 2003;9:263-268.
8. McEver RP. Selectin-carbohydrate interactions during inflammation and metastasis. *Glycoconj J* 1997;14:585-591.
9. Kannagi R. Carbohydrate-mediated cell adhesion involved in hematogenous metastasis of cancer. *Glycoconj J* 1997;14:577-584.
10. Borsig L, Stevenson JL, Varki A: Heparin in Cancer: Role of Selectin Interactions. In *Cancer-Associated Thrombosis* Edited by Khorana AA, Francis CW. New York: Informa Healthcare; 2007: 97-113.
11. Sallusto F, Lenig D, Forster R, Lipp M, Lanzavecchia A. Two subsets of memory T lymphocytes with distinct homing potentials and effector functions. *Nature* 1999;401:708-712.
12. Cines DB, Pollak ES, Buck CA, Loscalzo J, Zimmerman GA, McEver RP, et al. Endothelial cells in physiology and in the pathophysiology of vascular disorders. *Blood* 1998;91:3527-3561.
13. von Hundelshausen P, Weber C. Platelets as immune cells: bridging inflammation and cardiovascular disease. *Circ Res* 2007;100:27-40.

14. Borsig L. The role of platelet activation in tumor metastasis. *Expert Rev Anticancer Ther* 2008;8:1247-1255.
15. Sipkins DA, Wei X, Wu JW, Runnels JM, Cote D, Means TK, et al. In vivo imaging of specialized bone marrow endothelial microdomains for tumour engraftment. *Nature* 2005;435:969-973.
16. Morigi M, Zoja C, Figliuzzi M, Foppolo M, Micheletti G, Bontempelli M, et al. Fluid shear stress modulates surface expression of adhesion molecules by endothelial cells. *Blood* 1995;85:1696-1703.
17. Picker LJ, Kishimoto TK, Smith CW, Warnock RA, Butcher EC. ELAM-1 is an adhesion molecule for skin-homing T cells. *Nature* 1991;349:796-799.
18. Keelan ET, Licence ST, Peters AM, Binns RM, Haskard DO. Characterization of E-selectin expression in vivo with use of a radiolabeled monoclonal antibody. *Am J Physiol* 1994;266:H278-290.
19. Lowe JB. Glycan-dependent leukocyte adhesion and recruitment in inflammation. *Curr Opin Cell Biol* 2003;15:531-538.
20. Varki A. Selectin ligands: will the real ones please stand up? *J Clin Invest* 1997;99:158-162.
21. Rosen SD. Ligands for L-selectin: homing, inflammation, and beyond. *Annu Rev Immunol* 2004;22:129-156.
22. Sperandio M, Gleissner CA, Ley K. Glycosylation in immune cell trafficking. *Immunol Rev* 2009;230:97-113.
23. Katayama Y, Hidalgo A, Chang J, Peired A, Frenette PS. CD44 is a physiological E-selectin ligand on neutrophils. *J Exp Med* 2005;201:1183-1189.
24. Hidalgo A, Peired AJ, Wild MK, Vestweber D, Frenette PS. Complete identification of E-selectin ligands on neutrophils reveals distinct functions of PSGL-1, ESL-1, and CD44. *Immunity* 2007;26:477-489.
25. Picker LJ, Warnock RA, Burns AR, Doerschuk CM, Berg EL, Butcher EC. The neutrophil selectin LECAM-1 presents carbohydrate ligands to the vascular selectins ELAM-1 and GMP-140. *Cell* 1991;66:921-933.
26. Fuhlbrigge RC, King SL, Sackstein R, Kupper TS. CD43 is a ligand for E-selectin on CLA+ human T cells. *Blood* 2006;107:1421-1426.
27. Dimitroff CJ, Lee JY, Rafii S, Fuhlbrigge RC, Sackstein R. CD44 is a major E-selectin ligand on human hematopoietic progenitor cells. *J Cell Biol* 2001;153:1277-1286.
28. Kotovuori P, Tontti E, Pigott R, Shepherd M, Kiso M, Hasegawa A, et al. The vascular E-selectin binds to the leukocyte integrins CD11/CD18. *Glycobiology* 1993;3:131-136.
29. Steegmaier M, Levinovitz A, Isenmann S, Borges E, Lenter M, Kocher HP, et al. The E-selectin-ligand ESL-1 is a variant of a receptor for fibroblast growth factor. *Nature* 1995;373:615-620.
30. Hollingsworth MA, Swanson BJ. Mucins in cancer: protection and control of the cell surface. *Nat Rev Cancer* 2004;4:45-60.

31. Kim YJ, Varki A. Perspectives on the significance of altered glycosylation of glycoproteins in cancer. *Glycoconj J* 1997;14:569-576.
32. Aigner S, Sthoeger ZM, Fogel M, Weber E, Zarn J, Ruppert M, et al. CD24, a mucin-type glycoprotein, is a ligand for P-selectin on human tumor cells. *Blood* 1997;89:3385-3395.
33. Burdick MM, Chu JT, Godar S, Sackstein R. HCELL is the major E- and L-selectin ligand expressed on LS174T colon carcinoma cells. *J Biol Chem* 2006;281:13899-13905.
34. Gout S, Morin C, Houle F, Huot J. Death receptor-3, a new E-Selectin counter-receptor that confers migration and survival advantages to colon carcinoma cells by triggering p38 and ERK MAPK activation. *Cancer Res* 2006;66:9117-9124.
35. Dimitroff CJ, Descheny L, Trujillo N, Kim R, Nguyen V, Huang W, et al. Identification of leukocyte E-selectin ligands, P-selectin glycoprotein ligand-1 and E-selectin ligand-1, on human metastatic prostate tumor cells. *Cancer Res* 2005;65:5750-5760.
36. Läubli H, Borsig L. Heparins attenuate cancer metastasis: are selectins the link? *Cancer Invest* 2009;27:474-481.
37. Robinson SD, Frenette PS, Rayburn H, Cumiskey M, Ullman-Cullere M, Wagner DD, et al. Multiple, targeted deficiencies in selectins reveal a predominant role for P-selectin in leukocyte recruitment. *Proc Natl Acad Sci U S A* 1999;96:11452-11457.
38. Homeister JW, Zhang M, Frenette PS, Hynes RO, Wagner DD, Lowe JB, et al. Overlapping functions of E- and P-selectin in neutrophil recruitment during acute inflammation. *Blood* 1998;92:2345-2352.
39. Arbones ML, Ord DC, Ley K, Ratech H, Maynard-Curry C, Otten G, et al. Lymphocyte homing and leukocyte rolling and migration are impaired in L-selectin-deficient mice. *Immunity* 1994;1:247-260.
40. Mayadas TN, Johnson RC, Rayburn H, Hynes RO, Wagner DD. Leukocyte rolling and extravasation are severely compromised in P selectin-deficient mice. *Cell* 1993;74:541-554.
41. Labow MA, Norton CR, Rumberger JM, Lombard-Gillooly KM, Shuster DJ, Hubbard J, et al. Characterization of E-selectin-deficient mice: demonstration of overlapping function of the endothelial selectins. *Immunity* 1994;1:709-720.
42. Xia L, Sperandio M, Yago T, McDaniel JM, Cummings RD, Pearson-White S, et al. P-selectin glycoprotein ligand-1-deficient mice have impaired leukocyte tethering to E-selectin under flow. *J Clin Invest* 2002;109:939-950.
43. Maly P, Thall A, Petryniak B, Rogers CE, Smith PL, Marks RM, et al. The alpha(1,3)fucosyltransferase Fuc-TVII controls leukocyte trafficking through an essential role in L-, E-, and P-selectin ligand biosynthesis. *Cell* 1996;86:643-653.
44. Homeister JW, Thall AD, Petryniak B, Maly P, Rogers CE, Smith PL, et al. The alpha(1,3)fucosyltransferases FucT-IV and FucT-VII exert collaborative control over selectin-dependent leukocyte recruitment and lymphocyte homing. *Immunity* 2001;15:115-126.

45. Sperandio M, Thatte A, Foy D, Ellies LG, Marth JD, Ley K. Severe impairment of leukocyte rolling in venules of core 2 glucosaminyltransferase-deficient mice. *Blood* 2001;97:3812-3819.
46. Vestweber D. Regulation of endothelial cell contacts during leukocyte extravasation. *Curr Opin Cell Biol* 2002;14:587-593.
47. Ley K, Laudanna C, Cybulsky MI, Nourshargh S. Getting to the site of inflammation: the leukocyte adhesion cascade updated. *Nat Rev Immunol* 2007;7:678-689.
48. Zarbock A, Muller H, Kuwano Y, Ley K. PSGL-1-dependent myeloid leukocyte activation. *J Leukoc Biol* 2009;86:1119-1124.
49. Crockett-Torabi E. Selectins and mechanisms of signal transduction. *J Leukoc Biol* 1998;63:1-14.
50. Wong J, Johnston B, Lee SS, Bullard DC, Smith CW, Beaudet AL, et al. A minimal role for selectins in the recruitment of leukocytes into the inflamed liver microvasculature. *J Clin Invest* 1997;99:2782-2790.
51. Subramaniam M, Frenette PS, Saffaripour S, Johnson RC, Hynes RO, Wagner DD. Defects in hemostasis in P-selectin-deficient mice. *Blood* 1996;87:1238-1242.
52. Polgar J, Matuskova J, Wagner DD. The P-selectin, tissue factor, coagulation triad. *J Thromb Haemost* 2005;3:1590-1596.
53. Andre P, Denis CV, Ware J, Saffaripour S, Hynes RO, Ruggeri ZM, et al. Platelets adhere to and translocate on von Willebrand factor presented by endothelium in stimulated veins. *Blood* 2000;96:3322-3328.
54. Frenette PS, Denis CV, Weiss L, Jurk K, Subbarao S, Kehrel B, et al. P-Selectin glycoprotein ligand 1 (PSGL-1) is expressed on platelets and can mediate platelet-endothelial interactions in vivo. *J Exp Med* 2000;191:1413-1422.
55. Bone RC, Balk R, Slotman G, Maunder R, Silverman H, Hyers TM, et al. Adult respiratory distress syndrome. Sequence and importance of development of multiple organ failure. The Prostaglandin E1 Study Group. *Chest* 1992;101:320-326.
56. Brown KA, Brain SD, Pearson JD, Edgeworth JD, Lewis SM, Treacher DF. Neutrophils in development of multiple organ failure in sepsis. *Lancet* 2006;368:157-169.
57. Singbartl K, Green SA, Ley K. Blocking P-selectin protects from ischemia/reperfusion-induced acute renal failure. *FASEB J* 2000;14:48-54.
58. Mangell P, Mihaescu A, Wang Y, Schramm R, Jeppsson B, Thorlacius H. Critical role of P-selectin-dependent leukocyte recruitment in endotoxin-induced intestinal barrier dysfunction in mice. *Inflamm Res* 2007;56:189-194.
59. Tedder TF, Steeber DA, Pizcueta P. L-selectin-deficient mice have impaired leukocyte recruitment into inflammatory sites. *J Exp Med* 1995;181:2259-2264.
60. Norman KE, Cotter MJ, Stewart JB, Abbitt KB, Ali M, Wagner BE, et al. Combined anticoagulant and antiselectin treatments prevent lethal intravascular coagulation. *Blood* 2003;101:921-928.
61. Hansson GK. Inflammation, atherosclerosis, and coronary artery disease. *N Engl J Med* 2005;352:1685-1695.



62. Dong ZM, Brown AA, Wagner DD. Prominent role of P-selectin in the development of advanced atherosclerosis in ApoE-deficient mice. *Circulation* 2000;101:2290-2295.
63. Huo Y, Schober A, Forlow SB, Smith DF, Hyman MC, Jung S, et al. Circulating activated platelets exacerbate atherosclerosis in mice deficient in apolipoprotein E. *Nat Med* 2003;9:61-67.
64. Rivera-Nieves J, Burcin TL, Olson TS, Morris MA, McDuffie M, Cominelli F, et al. Critical role of endothelial P-selectin glycoprotein ligand 1 in chronic murine ileitis. *J Exp Med* 2006;203:907-917.
65. Collins RG, Velji R, Guevara NV, Hicks MJ, Chan L, Beaudet AL. P-Selectin or intercellular adhesion molecule (ICAM)-1 deficiency substantially protects against atherosclerosis in apolipoprotein E-deficient mice. *J Exp Med* 2000;191:189-194.
66. Galkina E, Kadl A, Sanders J, Varughese D, Sarembock IJ, Ley K. Lymphocyte recruitment into the aortic wall before and during development of atherosclerosis is partially L-selectin dependent. *J Exp Med* 2006;203:1273-1282.
67. Juliano RL. Signal transduction by cell adhesion receptors and the cytoskeleton: functions of integrins, cadherins, selectins, and immunoglobulin-superfamily members. *Annu Rev Pharmacol Toxicol* 2002;42:283-323.
68. Yoshida M, Westlin WF, Wang N, Ingber DE, Rosenzweig A, Resnick N, et al. Leukocyte adhesion to vascular endothelium induces E-selectin linkage to the actin cytoskeleton. *J Cell Biol* 1996;133:445-455.
69. Simon SI, Hu Y, Vestweber D, Smith CW. Neutrophil tethering on E-selectin activates beta 2 integrin binding to ICAM-1 through a mitogen-activated protein kinase signal transduction pathway. *J Immunol* 2000;164:4348-4358.
70. Ivetic A, Deka J, Ridley A, Ager A. The cytoplasmic tail of L-selectin interacts with members of the Ezrin-Radixin-Moesin (ERM) family of proteins: cell activation-dependent binding of Moesin but not Ezrin. *J Biol Chem* 2002;277:2321-2329.
71. Crockett-Torabi E, Sulenbarger B, Smith CW, Fantone JC. Activation of human neutrophils through L-selectin and Mac-1 molecules. *J Immunol* 1995;154:2291-2302.
72. Furukawa Y, Umemoto E, Jang MH, Tohya K, Miyasaka M, Hirata T. Identification of novel isoforms of mouse L-selectin with different carboxyl-terminal tails. *J Biol Chem* 2008;283:12112-12119.
73. Ivetic A, Ridley AJ. The telling tail of L-selectin. *Biochem Soc Trans* 2004;32:1118-1121.
74. Simon SI, Cherapanov V, Nadra I, Waddell TK, Seo SM, Wang Q, et al. Signaling functions of L-selectin in neutrophils: alterations in the cytoskeleton and colocalization with CD18. *J Immunol* 1999;163:2891-2901.
75. Blanks JE, Moll T, Eytner R, Vestweber D. Stimulation of P-selectin glycoprotein ligand-1 on mouse neutrophils activates beta 2-integrin mediated cell attachment to ICAM-1. *Eur J Immunol* 1998;28:433-443.

76. Weyrich AS, McIntyre TM, McEver RP, Prescott SM, Zimmerman GA. Monocyte tethering by P-selectin regulates monocyte chemotactic protein-1 and tumor necrosis factor- $\alpha$  secretion. Signal integration and NF- $\kappa$ B translocation. *J Clin Invest* 1995;95:2297-2303.
77. Wang HB, Wang JT, Zhang L, Geng ZH, Xu WL, Xu T, et al. P-selectin primes leukocyte integrin activation during inflammation. *Nat Immunol* 2007;8:882-892.
78. Evangelista V, Pamuklar Z, Piccoli A, Manarini S, Dell'elba G, Pecce R, et al. Src family kinases mediate neutrophil adhesion to adherent platelets. *Blood* 2007;109:2461-2469.
79. Urzainqui A, Serrador JM, Viedma F, Yanez-Mo M, Rodriguez A, Corbi AL, et al. ITAM-based interaction of ERM proteins with Syk mediates signaling by the leukocyte adhesion receptor PSGL-1. *Immunity* 2002;17:401-412.
80. Snapp KR, Heitzig CE, Kansas GS. Attachment of the PSGL-1 cytoplasmic domain to the actin cytoskeleton is essential for leukocyte rolling on P-selectin. *Blood* 2002;99:4494-4502.
81. Zarbock A, Abram CL, Hundt M, Altman A, Lowell CA, Ley K. PSGL-1 engagement by E-selectin signals through Src kinase Fgr and ITAM adapters DAP12 and FcR  $\gamma$  to induce slow leukocyte rolling. *J Exp Med* 2008;205:2339-2347.
82. Kufe DW. Mucins in cancer: function, prognosis and therapy. *Nat Rev Cancer* 2009;9:874-885.
83. Borsig L, Wong R, Hynes RO, Varki NM, Varki A. Synergistic effects of L- and P-selectin in facilitating tumor metastasis can involve non-mucin ligands and implicate leukocytes as enhancers of metastasis. *Proc Natl Acad Sci U S A* 2002;99:2193-2198.
84. Kim YJ, Borsig L, Varki NM, Varki A. P-selectin deficiency attenuates tumor growth and metastasis. *Proc Natl Acad Sci U S A* 1998;95:9325-9330.
85. Läubli H, Stevenson JL, Varki A, Varki NM, Borsig L. L-selectin facilitation of metastasis involves temporal induction of fut7-dependent ligands at sites of tumor cell arrest. *Cancer Res* 2006;66:1536-1542.
86. Rickles FR, Falanga A. Molecular basis for the relationship between thrombosis and cancer. *Thromb Res* 2001;102:V215-V224.
87. Wahrenbrock M, Borsig L, Le D, Varki N, Varki A. Selectin-mucin interactions as a probable molecular explanation for the association of Trousseau syndrome with mucinous adenocarcinomas. *J Clin Invest* 2003;112:853-862.
88. Khatib AM, Kontogiannea M, Fallavollita L, Jamison B, Meterissian S, Brodt P. Rapid induction of cytokine and E-selectin expression in the liver in response to metastatic tumor cells. *Cancer Res* 1999;59:1356-1361.
89. Brodt P, Fallavollita L, Bresalier RS, Meterissian S, Norton CR, Wolitzky BA. Liver endothelial E-selectin mediates carcinoma cell adhesion and promotes liver metastasis. *Int J Cancer* 1997;71:612-619.
90. Kannagi R. Molecular mechanism for cancer-associated induction of sialyl Lewis X and sialyl Lewis A expression-The Warburg effect revisited. *Glycoconj J* 2004;20:353-364.

91. Gout S, Tremblay PL, Huot J. Selectins and selectin ligands in extravasation of cancer cells and organ selectivity of metastasis. *Clin Exp Metastasis* 2008;25:335-344.
92. Burdick MM, McCaffery JM, Kim YS, Bochner BS, Konstantopoulos K. Colon carcinoma cell glycolipids, integrins, and other glycoproteins mediate adhesion to HUVECs under flow. *Am J Physiol Cell Physiol* 2003;284:C977-987.
93. Mannori G, Santoro D, Carter L, Corless C, Nelson RM, Bevilacqua MP. Inhibition of colon carcinoma cell lung colony formation by a soluble form of E-selectin. *Am J Pathol* 1997;151:233-243.
94. Fukuda MN, Ohyama C, Lowitz K, Matsuo O, Pasqualini R, Ruoslahti E, et al. A peptide mimic of E-selectin ligand inhibits sialyl Lewis X-dependent lung colonization of tumor cells. *Cancer Res* 2000;60:450-456.
95. Barthel SR, Wiese GK, Cho J, Opperman MJ, Hays DL, Siddiqui J, et al. Alpha 1,3 fucosyltransferases are master regulators of prostate cancer cell trafficking. *Proc Natl Acad Sci U S A* 2009;106:19491-19496.
96. Mannori G, Crottet P, Cecconi O, Hanasaki K, Aruffo A, Nelson RM, et al. Differential colon cancer cell adhesion to E-, P-, and L-selectin: role of mucin-type glycoproteins. *Cancer Res* 1995;55:4425-4431.
97. Aychek T, Miller K, Sagi-Assif O, Levy-Nissenbaum O, Israeli-Amit M, Pasmanik-Chor M, et al. E-selectin regulates gene expression in metastatic colorectal carcinoma cells and enhances HMGB1 release. *Int J Cancer* 2008;123:1741-1750.
98. Laferriere J, Houle F, Taher MM, Valerie K, Huot J. Transendothelial migration of colon carcinoma cells requires expression of E-selectin by endothelial cells and activation of stress-activated protein kinase-2 (SAPK2/p38) in the tumor cells. *J Biol Chem* 2001;276:33762-33772.
99. Tremblay PL, Auger FA, Huot J. Regulation of transendothelial migration of colon cancer cells by E-selectin-mediated activation of p38 and ERK MAP kinases. *Oncogene* 2006.
100. Biancone L, Araki M, Araki K, Vassalli P, Stamenkovic I. Redirection of tumor metastasis by expression of E-selectin in vivo. *J Exp Med* 1996;183:581-587.
101. Läubli H, Borsig L. Selectins as mediators of lung metastasis. *Cancer Microenviron* 2010;3:in press.
102. Honn KV, Tang DG, Crissman JD. Platelets and cancer metastasis: a causal relationship? *Cancer Metastasis Rev* 1992;11:325-351.
103. Karpatkin S, Pearlstein E. Role of platelets in tumor cell metastases. *Ann Intern Med* 1981;95:636-641.
104. Nash GF, Turner LF, Scully MF, Kakkar AK. Platelets and cancer. *Lancet Oncol* 2002;3:425-430.
105. Karpatkin S, Pearlstein E, Ambrogio C, Collier BS. Role of adhesive proteins in platelet tumor interaction in vitro and metastasis formation in vivo. *J Clin Invest* 1988;81:1012-1019.
106. Gasic GJ. Role of plasma, platelets, and endothelial cells in tumor metastasis. *Cancer Metastasis Rev* 1984;3:99-114.

107. Camerer E, Qazi AA, Duong DN, Cornelissen I, Advincula R, Coughlin SR. Platelets, protease-activated receptors, and fibrinogen in hematogenous metastasis. *Blood* 2004;104:397-401.
108. Borsig L, Wong R, Feramisco J, Nadeau DR, Varki NM, Varki A. Heparin and cancer revisited: mechanistic connections involving platelets, P-selectin, carcinoma mucins, and tumor metastasis. *Proc Natl Acad Sci U S A* 2001;98:3352-3357.
109. Ludwig RJ, Boehme B, Podda M, Henschler R, Jager E, Tandi C, et al. Endothelial P-selectin as a target of heparin action in experimental melanoma lung metastasis. *Cancer Res* 2004;64:2743-2750.
110. Qian F, Hanahan D, Weissman IL. L-selectin can facilitate metastasis to lymph nodes in a transgenic mouse model of carcinogenesis. *Proc Natl Acad Sci U S A* 2001;98:3976-3981.
111. Aviram R, Raz N, Kukulansky T, Hollander N. Expression of L-selectin and efficient binding to high endothelial venules do not modulate the dissemination potential of murine B-cell lymphoma. *Cancer Immunol Immunother* 2001;50:61-68.
112. Mantovani A, Allavena P, Sica A, Balkwill F. Cancer-related inflammation. *Nature* 2008;454:436-444.
113. Joyce JA, Pollard JW. Microenvironmental regulation of metastasis. *Nat Rev Cancer* 2009;9:239-252.
114. Coussens LM, Werb Z. Inflammation and cancer. *Nature* 2002;420:860-867.
115. Fidler IJ. The organ microenvironment and cancer metastasis. *Differentiation* 2002;70:498-505.
116. Qian B, Deng Y, Im JH, Muschel RJ, Zou Y, Li J, et al. A distinct macrophage population mediates metastatic breast cancer cell extravasation, establishment and growth. *PLoS One* 2009;4:e6562.
117. Chiang AC, Massague J. Molecular basis of metastasis. *N Engl J Med* 2008;359:2814-2823.
118. Pantel K, Brakenhoff RH. Dissecting the metastatic cascade. *Nat Rev Cancer* 2004;4:448-456.
119. Nierodzik ML, Karparkin S. Thrombin induces tumor growth, metastasis, and angiogenesis: Evidence for a thrombin-regulated dormant tumor phenotype. *Cancer Cell* 2006;10:355-362.
120. Ay C, Simanek R, Vormittag R, Dunkler D, Alguet G, Koder S, et al. High plasma levels of soluble P-selectin are predictive of venous thromboembolism in cancer patients: results from the Vienna Cancer and Thrombosis Study (CATS). *Blood* 2008;112:2703-2708.
121. Pantel K, Alix-Panabieres C, Riethdorf S. Cancer micrometastases. *Nat Rev Clin Oncol* 2009;6:339-351.
122. Cristofanilli M, Hayes DF, Budd GT, Ellis MJ, Stopeck A, Reuben JM, et al. Circulating tumor cells: a novel prognostic factor for newly diagnosed metastatic breast cancer. *J Clin Oncol* 2005;23:1420-1430.
123. Mueller BM, Reisfeld RA, Edgington TS, Ruf W. Expression of tissue factor by melanoma cells promotes efficient hematogenous metastasis. *Proc Natl Acad Sci U S A* 1992;89:11832-11836.
124. Ruf W, Mueller BM. Thrombin generation and the pathogenesis of cancer. *Semin Thromb Hemost* 2006;32 Suppl 1:61-68.

125. Clezardin P, Drouin J, Morel-Kopp MC, Hanss M, Kehrel B, Serre CM, et al. Role of platelet membrane glycoproteins Ib/IX and IIb/IIIa, and of platelet alpha-granule proteins in platelet aggregation induced by human osteosarcoma cells. *Cancer Res* 1993;53:4695-4700.
126. Oleksowicz L, Mrowiec Z, Schwartz E, Khorshidi M, Dutcher JP, Puszkin E. Characterization of tumor-induced platelet aggregation: the role of immunorelated GPIb and GPIIb/IIIa expression by MCF-7 breast cancer cells. *Thromb Res* 1995;79:261-274.
127. Trikha M, Zhou Z, Timar J, Raso E, Kennel M, Emmell E, et al. Multiple roles for platelet GPIIb/IIIa and alphavbeta3 integrins in tumor growth, angiogenesis, and metastasis. *Cancer Res* 2002;62:2824-2833.
128. Nieswandt B, Hafner M, Echtenacher B, Mannel DN. Lysis of tumor cells by natural killer cells in mice is impeded by platelets. *Cancer Res* 1999;59:1295-1300.
129. Palumbo JS, Talmage KE, Massari JV, La Jeunesse CM, Flick MJ, Kombrinck KW, et al. Tumor cell-associated tissue factor and circulating hemostatic factors cooperate to increase metastatic potential through natural killer cell-dependent and-independent mechanisms. *Blood* 2007;110:133-141.
130. Sawicki G, Salas E, Murat J, Miszta-Lane H, Radomski MW. Release of gelatinase A during platelet activation mediates aggregation. *Nature* 1997;386:616-619.
131. Belloc C, Lu H, Soria C, Fridman R, Legrand Y, Menashi S. The effect of platelets on invasiveness and protease production of human mammary tumor cells. *Int J Cancer* 1995;60:413-417.
132. Boucharaba A, Serre CM, Gres S, Saulnier-Blache JS, Bordet JC, Guglielmi J, et al. Platelet-derived lysophosphatidic acid supports the progression of osteolytic bone metastases in breast cancer. *J Clin Invest* 2004;114:1714-1725.
133. Vidal-Vanaclocha F, Fantuzzi G, Mendoza L, Fuentes AM, Anasagasti MJ, Martin J, et al. IL-18 regulates IL-1beta-dependent hepatic melanoma metastasis via vascular cell adhesion molecule-1. *Proc Natl Acad Sci U S A* 2000;97:734-739.
134. Alexiou D, Karayiannakis AJ, Syrigos KN, Zbar A, Kremmyda A, Bramis I, et al. Serum levels of E-selectin, ICAM-1 and VCAM-1 in colorectal cancer patients: correlations with clinicopathological features, patient survival and tumour surgery. *Eur J Cancer* 2001;37:2392-2397.
135. Matsuo Y, Amano S, Furuya M, Namiki K, Sakurai K, Nishiyama M, et al. Involvement of p38alpha mitogen-activated protein kinase in lung metastasis of tumor cells. *J Biol Chem* 2006;281:36767-36775.
136. Kobayashi K, Matsumoto S, Morishima T, Kawabe T, Okamoto T. Cimetidine inhibits cancer cell adhesion to endothelial cells and prevents metastasis by blocking E-selectin expression. *Cancer Res* 2000;60:3978-3984.
137. Auguste P, Fallavollita L, Wang N, Burnier J, Bikfalvi A, Brodt P. The host inflammatory response promotes liver metastasis by increasing tumor cell arrest and extravasation. *Am J Pathol* 2007;170:1781-1792.

138. Lu X, Kang Y. Chemokine (C-C motif) ligand 2 engages CCR2+ stromal cells of monocytic origin to promote breast cancer metastasis to lung and bone. *J Biol Chem* 2009;284:29087-29096.
139. Lewis CE, Pollard JW. Distinct role of macrophages in different tumor microenvironments. *Cancer Res* 2006;66:605-612.
140. Balkwill F, Charles KA, Mantovani A. Smoldering and polarized inflammation in the initiation and promotion of malignant disease. *Cancer Cell* 2005;7:211-217.
141. Hiratsuka S, Watanabe A, Aburatani H, Maru Y. Tumour-mediated upregulation of chemoattractants and recruitment of myeloid cells predetermines lung metastasis. *Nat Cell Biol* 2006;8:1369-1375.
142. Hiratsuka S, Nakamura K, Iwai S, Murakami M, Itoh T, Kijima H, et al. MMP9 induction by vascular endothelial growth factor receptor-1 is involved in lung-specific metastasis. *Cancer Cell* 2002;2:289-300.
143. Kaplan RN, Riba RD, Zacharoulis S, Bramley AH, Vincent L, Costa C, et al. VEGFR1-positive haematopoietic bone marrow progenitors initiate the pre-metastatic niche. *Nature* 2005;438:820-827.
144. Barthel SR, Gavino JD, Descheny L, Dimitroff CJ. Targeting selectins and selectin ligands in inflammation and cancer. *Expert Opin Ther Targets* 2007;11:1473-1491.
145. Marathe DD, Buffone A, Jr., Chandrasekaran EV, Xue J, Locke RD, Nasirikenari M, et al. Fluorinated per-acetylated GalNAc metabolically alters glycan structures on leukocyte PSGL-1 and reduces cell binding to selectins. *Blood* 2010;115:1303-1312.
146. Fuster MM, Brown JR, Wang L, Esko JD. A disaccharide precursor of sialyl Lewis X inhibits metastatic potential of tumor cells. *Cancer Res* 2003;63:2775-2781.
147. Brown JR, Fuster MM, Li R, Varki N, Glass CA, Esko JD. A disaccharide-based inhibitor of glycosylation attenuates metastatic tumor cell dissemination. *Clin Cancer Res* 2006;12:2894-2901.
148. Eppihimer MJ, Schaub RG. Soluble P-selectin antagonist mediates rolling velocity and adhesion of leukocytes in acutely inflamed venules. *Microcirculation* 2001;8:15-24.
149. Scalia R, Armstead VE, Minchenko AG, Lefer AM. Essential role of P-selectin in the initiation of the inflammatory response induced by hemorrhage and reinfusion. *J Exp Med* 1999;189:931-938.
150. Schon MP, Krahn T, Schon M, Rodriguez ML, Antonicek H, Schultz JE, et al. Efomycine M, a new specific inhibitor of selectin, impairs leukocyte adhesion and alleviates cutaneous inflammation. *Nat Med* 2002;8:366-372.
151. Kneuer C, Ehrhardt C, Radomski MW, Bakowsky U. Selectins--potential pharmacological targets? *Drug Discov Today* 2006;11:1034-1040.
152. Bock D, Philipp S, Wolff G. Therapeutic potential of selectin antagonists in psoriasis. *Expert Opin Investig Drugs* 2006;15:963-979.
153. Lever R, Page CP. Novel drug development opportunities for heparin. *Nat Rev Drug Discov* 2002;1:140-148.

154. Koenig A, Norgard-Sumnicht K, Linhardt R, Varki A. Differential interactions of heparin and heparan sulfate glycosaminoglycans with the selectins. Implications for the use of unfractionated and low molecular weight heparins as therapeutic agents. *J Clin Invest* 1998;101:877-889.
155. Stevenson JL, Choi SH, Varki A. Differential metastasis inhibition by clinically relevant levels of heparins--correlation with selectin inhibition, not antithrombotic activity. *Clin Cancer Res* 2005;11:7003-7011.
156. Ludwig RJ, Alban S, Bistran R, Boehncke WH, Kaufmann R, Henschler R, et al. The ability of different forms of heparins to suppress P-selectin function in vitro correlates to their inhibitory capacity on bloodborne metastasis in vivo. *Thromb Haemost* 2006;95:535-540.
157. Borsig L. Antimetastatic activities of modified heparins: selectin inhibition by heparin attenuates metastasis. *Semin Thromb Hemost* 2007;33:540-546.
158. Casu B, Vlodavsky I, Sanderson RD. Non-anticoagulant heparins and inhibition of cancer. *Pathophysiol Haemost Thromb* 2008;36:195-203.
159. Hostettler N, Naggi A, Torri G, Casu B, Vlodavsky I, Borsig L. P-selectin- and heparanase-dependent antimetastatic activity of non-anticoagulant heparins. *FASEB J* 2007;21:3562-3572.
160. Kuderer NM, Ortel TL, Francis CW. Impact of venous thromboembolism and anticoagulation on cancer and cancer survival. *J Clin Oncol* 2009;27:4902-4911.
161. Varki NM, Varki A. Heparin Inhibition of Selectin-Mediated Interactions during the Hematogenous Phase of Carcinoma Metastasis: Rationale for Clinical Studies in Humans. *Semin Thromb Hemost* 2002;28:53-66.
162. Kim MY, Oskarsson T, Acharyya S, Nguyen DX, Zhang XH, Norton L, et al. Tumor self-seeding by circulating cancer cells. *Cell* 2009;139:1315-1326.

## Figure legends:

### Figure 1 Selectin-mediated interaction in health and during cancer metastasis.

**a)** Selectins mediate initial interactions leading to adhesion of leukocytes and platelets to sites of locally activated endothelium often at sites of acute or chronic inflammation.

**b)** Potential interactions that occur between selectins and cancer cells during hematogeneous metastasis. All interactions have been shown to occur *in vivo*. While multiple interactions are possible, the temporal and spatial presence of selectins determines their contribution to metastasis. Potential role of selectins in metastasis: **1** – L-selectin mediates the recruitment of leukocytes to cancer cells, and L-selectin-mediated activation of leukocytes may be expected. Whether L-selectin mediates a direct contact between leukocytes and cancer cells remains to be determined. **2** – Platelet-cancer cell interactions are primarily mediated by P-selectin, and platelet aggregates. Thereby platelets protect cancer cells from the innate immune system and contribute to the means by which cancer cells adhere to and/or mechanistically arrest in the microvasculature. **3** – Adhesion of cancer cells to activated endothelium has been well described primarily *in vitro*. E- and P-selectin involvement in cancer cell adhesion to endothelium indicates a pro-metastatic function for activated endothelium *in vivo*. The mechanism leading to specific expression of E-selectin in distant organs remains to be defined.

### Figure 2 Selectins support tissue colonization by mediating heterotypic interactions within the metastatic microenvironment and the formation of a metastatic niche



a) Cancer cells from primary lesions reach blood vessels either directly or through lymph vessels. Intravasated cancer cells circulate until they become arrested in the microvasculature of distant organs. b) During circulation or after microvascular arrest, a tumor embolus is formed by P-selectin mediated interactions of tumor cells (TC) with platelets (minutes-hours after intravasation). The micrograph shows MC-38 tumor cells (grey) arrested in the pulmonary microvasculature 30 minutes after intravenous injection of tumor cells. Aggregates of platelets (red, CD41) are observed around the tumor cells. Nuclei are blue. EC, endothelial cells. c) Tumor embolus formation and intravascular arrest leads to the subsequent inflammatory activation of the metastatic microenvironment, including activation of endothelial cells (aEC) and recruitment of myeloid cells (MC) including neutrophils and monocytes (hours-days). Leukocyte recruitment depends partially on L-selectin and the expression of FucT VII dependent selectin ligands on activated endothelial cells. Moreover, endothelial P-selectin and E-selectin could be implicated in leukocyte recruitment. Selectin-mediated interactions contribute to local chemokine production that enhances inflammatory activation of the microenvironment. The micrograph shows myeloid cells (CD11b, green and Gr1, red positive cells) within the pulmonary microenvironment of intravenously injected MC-38 tumor cells (grey) during early lung colonization. Nuclei are blue. d) Inflammatory activation of the metastatic microenvironment partially mediated by selectins generates a metastatic niche, wherein tumor cells can survive, extravasate, proliferate and eventually form new metastatic lesions. The micrograph shows the growing edge of a metastatic lesion; tumor cells (green), F4/80 positive cells (red), nuclei (blue). From metastatic lesions, cancer cells can again intravasate and disseminate to other organs or the primary lesion [162].



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Figure 2

